exacerbated by elevated temperature. In summary, these results suggest temperature fluctuations could be a trigger for long-QT and Brugada related arrhythmias.

**1663-Pos Board B393**

**Trafficking Defective Mutations Modulate $Na_V1.5$ N—Glycosylation States**

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Voltage gated sodium channels are membrane proteins that play a critical role in electrical signaling of excitable cells. Amongst this family, the isoform $Na_v1.5$ is responsible for the initiation and propagation of cardiac action potentials. As most of membrane proteins, $Na_v1.5$ is well known to be a glycoprotein with ~5% of its total weight corresponding to carbohydrates. To date, it has been shown that $Na_v1.5$ glycosylations such as sialylations influence biophysical properties of this channel protein. However, whereas N-glycosylation are well known post-traductional modifications that modulate surface localization of many ionic channels, little is known about these maturation impacts on voltage gated sodium channels. Perturbation of $Na_v1.5$ trafficking is a well characterized phenomenon occurring in pathology such as Brugada syndrome. Our laboratory previously revealed that trafficking defective mutants of Nav1.5 exert a dominant negative effect upon wild type protein surface localization. The objective of this study was (i) to characterize N-glycosylations of $Na_v1.5$ during its membrane trafficking and (ii) to investigate these maturations in the context of the dominant negative effect exerted by $Na_v1.5$ trafficking defective mutations.

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**Selective Inhibition of Late $Na^+$ Current Reduces Arrhythmic Activity in Spontaneously Hypertensive Rat Myocytes**

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The cardiac late $Na^+$ current ($I_{NaL}$) is enhanced in hypertrophy, heart failure and ischemia. Spontaneously hypertensive rats (SHR) are characterized by prolonged action potential duration (APD), $[Na^+]_i$ and $[Ca^{2+}]_i$ overload and triggered activity (TA). We hypothesized that $I_{NaL}$ is enhanced in SHR and contribute to APD prolongation and TA. $I_{NaL}$ was increased in ventricular myocytes isolated from 10- to 12-month-old SHR ($0.84 \pm 0.13$ pA/pF, $n = 16$) compared to age-matched Wistar rats (WR; $0.26 \pm 0.06$ pA/pF, $n = 9$; $p < 0.01$). $I_{NaL}$ inhibitors ranolazine (RAN) and GS-458967 (GS967) reduced $I_{NaL}$ in myocytes from SHR with $IC_{50}$ of $3.7 \pm 0.1$ and $0.65 \pm 0.04$ μM; respectively. $APD_{70}$ and $APD_{90}$ were prolonged in myocytes from SHR, compared to WR, and were shortened by RAN (10 μM) and GS967 (1 μM) (Table). In 8/23myocytes from SHR, spontaneous delayed afterpotentials (DADs) and/or TA were observed. Pacing induced DADs in 8/10 and TA in 4/10 myocytes from SHR. GS967 (1 μM) abolished both spontaneous and pacing induced TA (4/4).Our data show that $I_{NaL}$ is enhanced and contributes to APD prolongation and TA in myocytes from SHR. Selective inhibition of $I_{NaL}$ could stabilize cardiac repolarization and suppress arrhythmias in hypertension.

Table. Action potential duration

| | $APD_{70}$ (msec) | $APD_{90}$ (msec) | n |
|---|---|---|---|
| WR | 34.6±3.4 | 61.8±4.0 | 22 |
| SHR | 58.6±7.0* | 95.5±6.6* | 7 |
| SHR + RAN | 50.0±7.3† | 85.4±8.9† | 4 |
| SHR + GS967 | 43.4±9.9† | 74.6±12.0† | 4 |

*P<0.05 vs. WR unpaired *t*-Test, †P<0.05 vs. SHR, paired *t*-Test
$APD_{70}$ and $APD_{90}$: APD at 70 and 90% repolarization.

**1665-Pos Board B395**

**Dynamic Late Na Current During Cardiac Action Potential Revealed by a Specific and Potent Inhibitor Gs967**

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The late $Na^+$ current (INa,L) is known to contribute to cardiac action potential (AP) plateau and upregulation of INaL leads to arrhythmogenic activities. We used an innovative self-action potential (sAP)-clamp technique to directly record the dynamic INa,L during the cell's own AP. We studied the dynamic INaL in rabbit ventricular myocytes under physiological conditions wherein $[Na^+]$i and $[Ca^{2+}]$i homeostasis were preserved. We then determined the effect of GS967, a newly developed selective and potent inhibitor of INa,L. Results: (1) During the various phases of AP waveform, the dynamic INa,L amplitude was low at phase-1, gradually increased during phase-2 to reach a peak, and declined at phase-3. This profile of INa,L magnitude and time course under AP-clamp is distinctively different from the monotonically declining INa,L current seen under rectangular pulse voltage-clamp due to non-equilibrium gating. (2) GS967 selectively inhibits INa,L during AP in a concentration-dependent manner with an IC50 of 57 nM. Unlike tetrodotoxin, GS967 did not significantly inhibit the fast $Na^+$ current. Hence, GS967 shortened the AP duration without affecting the AP upstroke. (3) The INa,L peak amplitude was $0.78 \pm 0.07$ A/F under physiological condition. Anemonia toxin II (ATX-II) at 5 nM increased the INaL amplitude to $1.25 \pm 0.14$ A/F, and prolonged APD95 from $212.4 \pm 10.5$ ms (control) to $305.6 \pm 19.1$ ms (ATX-II). GS967 (1uM) effectively shortened APD and suppressed afterdepolarizations (EADs) induced by ATX-II. Conclusion: Our sAP-clamp data reveal a surprisingly large INa,L during AP plateau under physiological condition, which explains why INa,L significantly affect cardiac AP morphology and arrhythmogenesis. GS967 inhibits INa,L during AP plateau without blocking the fast $Na^+$ current at AP upstroke, and therefore provides a promising therapeutic strategy to suppress cardiac arrhythmias without slowing conduction.

**1666-Pos Board B396**

**Increased Activity of Nav1.9 Sodium Channels Causes Loss of Pain Perception**

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Nociceptive sensory neurons transmit painful stimuli from the periphery to the central nervous system. Voltage-gated sodium (NaV) channels are instrumental for the generation of the corresponding electrical signals, but - so far - the sensory neuron-specific NaV1.9 only appeared to play a minor role. Recently, we identified a de novo heterozygous mutation in the SCN11A gene encoding for NaV1.9 and demonstrated the implication of NaV1.9 in human pain perception (Leipold et al., Nature Genetics, DOI 10.1038/ng.2767). Surprisingly, in affected individuals the mutation (L811P) leads to an inability to experience pain by conferring gain-of-function (GOF) properties to NaV1.9. Mutant channels activate at hyperpolarized voltages and display a slow-down of channel inactivation and deactivation, thereby causing sustained depolarization of nociceptor cells and alterations of action potential characteristics. This new channelopathy is different from other pain-related NaV channel disorders: a GOF of homologous NaV1.7 channels is associated with chronic pain, and loss of functional NaV1.7 channels causes congenital indifference to pain. To gain further insight into the mechanisms underlying the mutation we introduced the homologous L-to-P mutation into channel isoforms NaV1.4 (L802P), NaV1.7 (L957P) and NaV1.8 (L890P) and compared the functional parameters of wild-type and mutant channels by means of the whole-cell patch-clamp technique. The L-to-P mutation shifted the activation in all channel subtypes to hyperpolarized potentials in the order NaV1.9 > NaV1.7 > NaV1.4 > NaV1.8. Fast channel inactivation was slowed down most prominently in NaV1.9 and NaV1.8, followed by NaV1.7 and NaV1.4. These results show that a homologous L-to-P mutation affects channel activation and inactivation in a subtype-specific manner.

**1667-Pos Board B397**

**Epilepsy Increases the Contribution of Tetrodoxin-Sensitive Channels to the Cardiac Sodium Current**

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Human mortality associated with *status epilepticus* (SE) in U.S and Canada is 21%-22%. Sudden death caused by epilepsy (SUDEP) accounts for 17% of all SE-related casualties. Clinical evidence linked SUDEP to arrhythmias such as conduction block and prolongation of the QT interval, an index of repolarization time, on the cardiac electrocardiogram. These observations suggest that SE altered the activity of the cardiac sodium current ($I_{Na}$) involved in both cardiac conduction and repolarization of the ventricle. We tested this hypothesis by characterizing $I_{Na}$ in ventricular cardiomyocytes of epileptic rats induced induced by kainic acid injection. Our patch clamp results show that epilepsy increased peak $I_{Na}$ by $18 \pm 6\%$ and its sustained (late) component ($I_{NaL}$) by $53 \pm 13\%$. Activation of $I_{Na}$ occurred at more negative potential in epileptic rats and recovery from activation was significantly delayed by epilepsy. Because epilepsy is known to increase the expression of neuronal channels (nNaVs) in the brain we next tested if the changes in $I_{Na}$ were associated with increased sensitivity to tetrodotoxin (TTX), a hallmark of nNaVs. Our results indicate that TTX at a concentrations not affecting the cardiac sodium channel NaV1.5 (1 nM) blocked 34% and 50% of $I_{NaL}$ in Sham and epileptic rats respectively. Epilepsy therefore increased the contribution of neuronal sodium channel to $I_{Na}$ in the cardiac ventricle. In summary, our data indicate that epilepsy altered $I_{Na}$ in a manner consistent with the alterations of